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APPLICATION NUMBER: 60/513,040
FILING DATE: *October 21, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/34761*

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Title: **SYSTEM AND METHOD FOR THE
TREATMENT OF BRAIN TUMORS**

Atty. Dkt. PW 81476-306470
M#

Wheeler et al.
Client Ref

including:

Date: October 21, 2003

1. Specification: 24 pages 1A. ☒ Claim: 1 pages 1B ☒ 1 Abstract pages
2. ☐ Specification in non-English language 3. ☒ Drawings: 5 sheet(s)

4. The invention ☐ was ☒ was not made by, or under a contract with, an agency of the U.S. Government.
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6. Small Entity Status ☒ is Not claimed ☒ is claimed (pre-filing confirmation required)
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8. This application is made by the following named inventor(s) (Double check instructions for accuracy.):

(1) Inventor	Christopher	J	WHEELER
	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship

(2) Inventor	Asha		DAS
	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship

(3) Inventor	Keith	L	BLACK
	First	Middle Initial	Family Name
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Pillsbury Winthrop LLP
Intellectual Property Group
725 South Figueroa Street Suite 2800
Los Angeles, CA 90017-5406
Tel: (213) 488-7100
Atty/Sec: SDL/SBK

By Atty: Seth D. Levy **Reg. No.** 44,869

Sig:  **Fax:** (213) 629-1033
Tel: (213) 488-7100

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Appln. No.: Unassigned

Examiner: Unassigned

Filed: October 21, 2003

Atty Dkt: 81476-306470

C# M#

Title: **SYSTEM AND METHOD FOR THE TREATMENT
OF BRAIN TUMORS**

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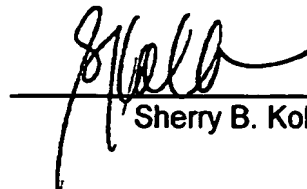
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Sherry B. Kolber

**APPLICATION FOR A
PROVISIONAL UNITED STATES PATENT
IN THE NAMES OF**

CHRISTOPHER J. WHEELER, ASHA DAS AND KEITH L. BLACK

FOR

SYSTEM AND METHOD FOR THE TREATMENT OF BRAIN TUMORS

ASSIGNED TO

CEDARS-SINAI MEDICAL CENTER

ATTORNEY DOCKET NO. 81476-306470

**PILLSBURY WINTHROP LLP
725 South Figueroa Street, Suite 2800
Los Angeles, California 90017-5406
Telephone: (213) 488-7100
Facsimile: (213) 629-1033**

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INTRODUCTION

Malignant brain tumors are among the gravest forms of cancer. The most common of these incurable tumors, glioblastoma multiforme (GBM), is responsible for 50% of all intracranial gliomas and 25% of intracranial tumors in adults (1, 2). GBM diagnosis carries with it an average survival between 12 and 18 months (with 90-95% patients surviving less than 2 years), without the possibility of spontaneous remission or effective treatment (1-3). The consistently short survival and absence of spontaneous remission that makes GBM such a devastating disease also renders the evaluation of new therapies for this disease relatively rapid and unequivocal. Overall survival represents the standard by which therapies for GBM are evaluated, in part because tumor mass reduction (i.e., surgically) does not necessarily correlate with prolonged survival (4-6).

Unfortunately, conventional therapies are remarkably ineffective at improving GBM clinical outcome despite their ability to confer significant benefits to patients with non-glioma tumors (3, 7, 8). Even the few treatments effective against GBM typically either exhibit small increases in survival that are evident only from large population studies, or primarily benefit certain (i.e., young) patient subpopulations (9, 10). Thus, novel therapies for GBM are needed.

Cancer vaccines represent one novel therapy for GBM (11-13). The clinical efficacy of therapeutic vaccination for any human tumor, however, remains controversial because consistent tumor destruction or extended lifespan is not observed in most vaccinated cancer patients (14-16). In contrast, current cancer vaccines do reliably elicit tumor-reactive cytotoxic T lymphocytes (CTL) in most patients (14, 15, 17). The reasons underlying the general clinical failure of cancer vaccines are unknown, but one possibility is that the kinetics of anti-tumor CTL killing in cancer patients may be too inefficient to keep pace with rapidly growing, mutating tumors in situ. Consistent with this notion, it was previously reported that therapeutic vaccination with autologous tumor antigen-pulsed dendritic cells is sufficient to enhance peripheral tumor-reactive CTL activity and CD8⁺ T cell infiltration into tumors in situ in GBM patients (13). Nevertheless, improvements in overall patient survival were not apparent in this initial study.

Since CTL induce death in their cellular targets, it is not unreasonable to expect that inefficient CTL killing might either incompletely trigger death pathways in targeted tumor cells, or select for CTL-resistant tumor variants. In the first case, vaccine-elicited tumor-responsive CTL might fundamentally alter tumors by “priming” their death machinery. In the second case, such CTL could fundamentally alter tumor cell physiology and/or genetics. Both of these possibilities could in theory be exploited by additional therapeutic modalities. Therefore, the clinical insufficiency of cancer vaccines encourages the examination of synergy between vaccination and other therapies.

A retrospective examination of the impact of therapeutic vaccination on the efficacy of conventional GBM chemotherapy was thus undertaken. Progression rates and overall survival was compared among 12 vaccine-treated, 13 chemotherapy-treated, and 13 vaccine plus chemotherapy-treated de novo GBM patients. The results suggest that chemotherapy synergizes with previous therapeutic vaccination to generate a uniquely effective treatment that slows GBM progression and significantly extends patient survival relative to individual therapies. This represents the first evidence that a vaccine-based therapeutic approach may benefit a majority of cancer patients, and potentially represents a novel treatment strategy that may substantially prolong GBM survival across a wide age range and relative to standard radiation plus chemotherapy. Additional independent evidence implicated anti-tumor T cells as influencing GBM chemosensitivity.

METHODS

Patients & clinical parameters

All patients suffered from newly-diagnosed GBM (55 yrs avg, 32-78 range) and provided informed consent to treatments and associated monitoring.

Patients in the “vaccine group” underwent craniotomy (5 patients underwent one craniotomy prior to receiving vaccine therapy, 6 underwent two craniotomies, and one patient underwent four craniotomies prior to receiving vaccine therapy). All of these patients received a course of radiation prior to vaccination. Four patients in this group also received chemotherapy and 1 patient received stereotactic radiosurgery (SRS) prior to vaccination. After vaccination 5 of these patients underwent another craniotomy and 3 received additional SRS. None received chemotherapy following vaccination.

All patients in the “chemotherapy group” underwent craniotomy, radiation, and chemotherapy. Six of these patients underwent a second craniotomy and 5 patients received additional SRS. Of note, the longest overall survivor in this group (991 days) suffered from post-operative intracranial abscess requiring multiple surgical procedures for drainage. Intracranial infections in malignant glioma patients are associated with prolonged survival and have been proposed to initiate an anti-tumor immune response (18).

Patients in the “vaccine and chemotherapy group” underwent craniotomy (8 patients underwent one craniotomy and 5 underwent two craniotomies) prior to receiving the vaccine therapy. All of these patients received radiation therapy. Five patients received additional chemotherapy and 3 received SRS. Following vaccination, 6 of these patients underwent another craniotomy and 5 received SRS. All patients received chemotherapy following vaccination at the time of tumor progression. Notably, a single patient in this group (surviving > 730 days and depicted in Fig. 3B) experienced a cutaneous glioblastoma with single lymph node involvement prior to vaccination and at the site of irradiated tumor cell inoculation for DTH testing. These two tumors were removed surgically approximately 1 year prior to chemotherapy and did not recur.

Vaccinated patients were steroid-free during blood collection and vaccinations as described (13), and received 3 vaccines, two weeks apart, of $10\text{--}40 \times 10^6$ autologous dendritic cells loaded with either HLA-

eluted peptides from cultured tumor cells, or 150 µg/ml autologous tumor freeze-thaw lysate, starting approximately 15 weeks post-surgery. A fourth identical vaccination followed 6 weeks later only in phase II trial patients (12 of 25). Serial MRI scans were performed every 2 to 3 months for all patients.

Cell isolation & lysis

PBMC were prepared with Ficoll from patients' blood obtained at the time of surgery and/or from banked leukaphereses. CD4⁺ and CD8⁺ T cells were purified from PBMC using MACS bead separation (Milenyi Biotec, Auburn, CA). 10⁷ CD4⁺ or CD8⁺ cells/ml were prepared for qPCR by lysis in 100 µg/mL proteinase K (Boehringer, Indianapolis, IN) 1 hr, 56°C, with inactivation at 95°C, 10 min.

TREC quantification

TRECs were quantified in duplicate or triplicate by quantitative real-time PCR (qPCR) using the 5' nuclease (TaqMan) method as previously described (19), and detected on an iCycler system (BioRad, Hercules, CA). qPCR was performed on 5 µL cell lysate (from 50,000 cells) with primers: 5'-CACATCCCTTTCAACCATGCT-3' (forward), 5'-GCCAGCTGCAGGGTTTAGG-3' (reverse), and FAM-5'-ACACCTCTGGTTTTTGTAAGGTGCCCACT-TAMRA-3' (probe; MegaBases, Chicago, IL). PCR reactions including 0.5 U Platinum Taq (Gibco, Grand Island, NY), 3.5 mM MgCl₂, 0.2 mM dNTPs, 500 nM of each primer, 150 nM probe, were amplified at 95°C for 5 min, 95 °C for 30 sec, and 60 °C for 1 min for 45 cycles. Control β-actin reactions were performed to ensure nucleic acid content and negative samples were excluded from further analysis. TREC values were adjusted for T cell purity.

Statistical analyses

Statistical analyses included 2-tailed Mann-Whitney log-rank tests for disease-free and overall survival, binomial distribution probability, and Pearson's correlation coefficients (r values) calculated with SAS and Excel software. Binomial distributions were determined for 2-year and 3-year survival frequencies between vaccine + chemotherapy and other (vaccine or chemotherapy) patient groups.

RESULTS

Newly-diagnosed de novo GBM patients (GBM did not arise from malignant progression of initially lower-grade gliomas) were enrolled into one of three vaccine studies conducted from 1998-2001 at a single institution (MDNSI), or were administered chemotherapy alone, after surgical tumor resection and standard radiation therapy. Vaccinated (“vaccine” or “vaccine + chemotherapy”) patients received at least 3 vaccinations with autologous tumor antigen-pulsed dendritic cells, starting approximately 15 weeks post-surgery and 5 weeks post-radiation therapy (Tables I and II). Patients receiving chemotherapy alone (“chemotherapy” patients) were treated (with surgery, radiation and chemotherapy) over the same time interval as vaccinated patients (Table III). Serial MRI scans were performed every 2-3 months in all patients. Tumor progression and overall survival among vaccine, chemotherapy and vaccine + chemotherapy groups was determined and compared (Fig. 1 and 2).

Treatment and demographic parameters, including extent of non-survivors, surgery before or after vaccination (where appropriate), chemotherapy before vaccination (where appropriate), gender and age were not significantly different among the relevant groups (Table I). In addition, Karnofsky performance status (KPS), was not significantly different between vaccine and vaccine + chemotherapy patient groups (Table I). Inclusion criteria for vaccinated de novo GBM patients, the patient subgroup common among the 3 vaccine trials, were identical in the 3 vaccine trials (Table II). Similarly, anti-tumor immune response rates were similar for the 3 vaccine trials (Table II), suggesting that differences in antigen source and/or vaccine dosing among individual vaccine trials did not substantially impact their immunological efficacy. Moreover, vaccine, vaccine + chemotherapy, and chemotherapy patients exhibited identical recurrence times following vaccination (Fig. 1), indicating a lack of inherent bias in tumor clinical behavior among all three groups after initial treatment. For these reasons, and despite the fact that the trials were not designed to address the issue, we felt that analysis of these patient groups could reveal testable hypotheses pertaining to synergy between vaccination and chemotherapy.

Only the extent of surgical resection differed significantly between the patient groups, with all vaccinated patients receiving image-complete resections and a portion of chemotherapy patients receiving partial resections. This particular bias was expected to produce longer survival selectively in vaccine relative to chemotherapy groups. On the contrary, mean times to progression and overall survival of chemotherapy patients was similar to those in previous reports (8) and did not differ significantly from those of vaccinated patients (Table II; Fig. 1, 2). Thus, therapeutic vaccination by itself failed to significantly slow progression or prolong survival relative to conventional GBM chemotherapy ($P = 0.7$, log-rank). These results suggested that anti-tumor immunity was either inefficiently induced or GBM tumors were inherently resistant to vaccine-elicited immune destruction alone. Since efficient induction of anti-tumor CTL responses by our vaccine methodology was previously demonstrated (13), the latter possibility appeared most likely.

To determine if vaccination was capable of eliciting more subtle affects on GBM tumors, we examined whether vaccination could alter GBM sensitivity to subsequent chemotherapy. GBM patients receiving chemotherapy after vaccination enjoyed significantly prolonged tumor progression relative to those receiving vaccination or chemotherapy alone. Similarly, GBM patients receiving chemotherapy after vaccination exhibited significantly prolonged survival relative to those receiving either treatment individually. The possibility that an inadvertent selection bias resulted in inherently slower progressing tumors in patients receiving chemotherapy after vaccination is inconsistent with the statistically identical initial progression times (i.e., after initial vaccination or chemotherapy) among all three groups of patients ("vaccine", "chemotherapy" and "vaccine + chemotherapy"): tumors behaved clinically the same regardless of initial vaccination or chemotherapy and slowed tumor progression was unique to the period of chemotherapy after vaccination. In addition, the two groups of vaccinated patients were statistically identical in terms of all other treatment parameters, including number of craniotomies, radiation, stereotactic radiosurgery and chemotherapy prior to vaccination, and exhibited similar Karnofsky performance scores after vaccine therapy.

Importantly, 2-, 3- and 4-year survival was also unique for patients receiving chemotherapy after vaccination. Whereas chemotherapy or vaccination alone resulted in 2-year survival within the established range for GBM (8%; Table I), post-vaccination chemotherapy resulted in a substantial increase (42%; Table I) in 2-year survivors ($P < 0.05$; binomial distribution). Similarly, no 3-year or 4-year survivors were evident after chemotherapy or vaccination alone, but such survivors persisted in post-vaccine chemotherapy patients ($P < 0.01$ for 3-year survivors; binomial distribution).

Finally, objective ($> 50\%$) regression of the entire tumor mass was observed in 3 of 13 vaccine + chemotherapy patients and this occurred only after initiation of post-vaccine chemotherapy. A similar regression was also observed in a single grade III malignant glioma patient receiving chemotherapy after vaccination (data not shown). Such dramatic regression of de novo GBM was unique to this group and is unknown in the literature, although a single example of partial regression in a recurrent GBM following post-vaccine chemotherapy has recently been reported (20). In that report, rapid tumor recurrence, as judged by increased tumor on imaging studies, clearly occurred following vaccination, but was discounted to favor the notion that regression was related to vaccination itself. In this context, it is significant that tumor recurrence in all vaccinated patients in the current study was determined by increased tumor imaging on MRI scans. Moreover, 33% (4/12) of vaccine patients and 46% (6/13) of vaccine + chemotherapy patients were biopsied upon observation of post-vaccine increases in tumor imaging, with all exhibiting histologically verified recurrent tumor. This suggests that apparent increases in tumor imaging in our study were not due to vaccine-induced inflammatory responses and instead generally reflected bona fide tumor recurrence. This suggests that the specific therapeutic regimen of chemotherapy after vaccination, rather than vaccination alone, elicited the tumor regressions. In any case, this is the first demonstration of objective regression of entire tumor mass in any adoptive immunotherapy setting, as well as in the treatment of GBM generally.

The above results allowed us to hypothesize that clinical outcome is significantly improved by the specific combination and sequence of vaccination plus chemotherapy in GBM patients. Stated more generally, we hypothesized that anti-tumor immunity directly impacts GBM chemosensitivity. Although

the concern over inadvertent bias due to patient selection was substantially reduced by empirical demonstration of initially identical tumor behavior among the three patient groups analyzed, independent means of testing this hypothesis were nonetheless desired.

Thymic production of CD8⁺ T cells, which is accurately reflected by the concentration of T cell receptor excision circles (TRECs) in purified T cells (19, 21, 22), accounts for age-dependent glioma prognosis and outcome and predominantly influences vaccine-induced anti-tumor responses in GBM patients (23). We surmised that a direct influence of anti-tumor immunity on GBM chemosensitivity, itself an age-dependent phenomenon (9), would be reflected by a dominant relationship between CD8⁺ TRECs and chemotherapeutic responsiveness within the same GBM patients. Accordingly, TREC content within purified CD8⁺ T cells dominantly correlated with the increase in tumor recurrence times following post-vaccine chemotherapy. This relationship was not simply a function of an independent influence of age on chemosensitivity and thymic production of CD8⁺ T cells, because the strength and significance of this correlation surpassed that between increased recurrence times following post-vaccine chemotherapy and patient age (Fig. 4).

The close relationship between thymus products and glioma outcome is a direct result of CD8⁺ T cell production and/or function (23). Thus, the dominant relationship between CD8⁺ TRECs and prolonged progression times following post-vaccine chemotherapy suggests that clinical responsiveness to chemotherapy is similarly impacted by production and/or function of newly emigrated CD8⁺ T cells. Since levels of such T cells were shown to predominantly mediate anti-tumor immune responsiveness following vaccination of GBM patients (23), this constitutes independent validation of the hypothesis that anti-tumor immunity impacts GBM chemosensitivity.

Taken together, these findings suggest that GBM tumors are recognized and acted on in situ by cellular immune components. Overall, such activity may result in fundamental alteration of GBM tumors that renders them increasingly susceptible to DNA damaging chemotherapy, despite the inability of vaccination by itself to confer overt clinical benefits to patients

DISCUSSION

Although originating from distinct clinical studies not designed to address synergy between vaccination and chemotherapy, several criteria help validate comparison among the three patient treatment groups and, especially, between the two vaccine patient groups. First, the overwhelming prognostic factors for glioma clinical outcome are tumor type, tumor grade and patient age. All three groups were comprised only of patients with identical types and grades of tumor (grade IV astrocytoma = glioblastoma multiforme), and exhibited statistically identical age ranges. In addition, a less robust and more subjective prognostic factor, Karnofsky performance status, which was not followed rigorously in chemotherapy patients, was not statistically different between vaccine and vaccine plus chemotherapy patients after vaccination. This suggests that, among vaccinated patients, there was no bias in a prognostic factor that was obtained near the time of segregation into vaccine only or vaccine plus chemotherapy subgroups. Finally, and perhaps most convincingly, all three groups exhibited identical tumor recurrence times after initial therapy (vaccination or chemotherapy), suggesting that tumors from patients in any one group behaved clinically like those from any other. Thus, no prognostically or empirically defined bias in patient composition or tumor behavior among the three treatment groups was evident prior to secondary chemotherapy.

A potential bias between chemotherapy and all vaccinated patients was, however, possible due to a portion of chemotherapy patients experiencing partial surgical resection of their tumors, whereas all patients receiving vaccinations had gross total resections. Although the clinical benefit of complete surgical resection has not been scientifically addressed by design, it is thought to provide a statistically significant survival increase (24). This increase is minimal, however, and has only been evident from meta-analyses of hundreds of patients in any study. We therefore do not expect a proportion of incompletely resected patients within an already limited population to account for the relatively large difference in survival between chemotherapy and vaccine plus chemotherapy patients. This expectation is supported by the fact that survival was statistically identical between chemotherapy and vaccine

(without subsequent chemotherapy) patient groups, the latter of which, like the vaccine plus chemotherapy group, was comprised entirely of patients with gross total tumor resections. Thus, the only obvious potential bias in patient composition among the three treatment groups is statistically unlikely and directly refuted by empirical clinical data. We therefore felt justified in comparing tumor progression and overall survival among three treatment groups of de novo GBM patients with identical prognostic and pre-treatment factors.

Vaccinated patients receiving subsequent chemotherapy exhibited significantly delayed tumor progression and longer survival relative to those receiving vaccinations without subsequent chemotherapy or to those receiving chemotherapy alone. Improved clinical outcome appeared dependent on the specific combination and sequence of therapeutic vaccination followed by chemotherapy.

These observations suggest a substantial therapeutic slowing of GBM progression and extension of overall survival for GBM patients. These clinical benefits appeared to markedly surpass those in previous vaccine studies as well as those in even the most hopeful analyses of GBM chemotherapy (8). Moreover, the more favorable clinical outcome conferred by post-vaccine chemotherapy did not appear to be confined to younger subgroups of patients. As such, this allows us to hypothesize that this specific treatment combination conferred clinical improvement to a majority of treated cancer patients, which, if further substantiated, would be unique for a vaccine-based therapy. A controlled prospective analysis is thus warranted to rigorously test the prediction that combinatorial immune/chemotherapy is superior to either vaccine therapy or standard chemotherapy alone and represents the best available treatment for GBM.

It has been unclear whether the failure of therapeutic cancer vaccines for most patients stems from the prevention of vaccine-elicited CTL responsiveness to tumor cells or from the inability of responding CTL to counteract net tumor expansion (17). The results presented here are consistent with the sufficiency of current DC-based vaccines to elicit fundamental physiological changes in GBM tumors, manifested as sensitivity to chemotherapy, while failing to cause net tumor destruction in the absence of additional treatment. While this does not exclude the possibility that anti-GBM CTL responsiveness is

mutated in GBM patients a priori, it raises the additional possibility that net tumor destruction may be impeded subsequent to CTL anti-tumor responses. In any case, CTL activity may tangibly alter GBM tumors such that substantial tumor destruction, as evidenced by slowed tumor progression and prolonged patient survival, may be elicited by the additional induction of DNA damage through post-vaccine chemotherapy.

Both clinical outcome and chemotherapeutic responsiveness are known to be age-dependent processes in gliomas generally (3, 9, 25). Age-dependent glioma clinical outcome is critically impacted by the production of CD8⁺ T cells in the thymus in mice and a directly related parameter, T cell receptor excision circle (TREC) concentration within CD8⁺ T cells, accounts entirely for age-dependent prognosis in GBM patients (23). This raises the possibility of a similar immune impact on additional age-dependent properties of GBM, such as responsiveness to chemotherapy. In support of this, we observed a robust correlation between CD8⁺ TRECs and GBM responsiveness to post-vaccine chemotherapy that surpassed that between age and responsiveness to chemotherapy. Thus, a particular cellular immune parameter appears to not only account for the strongest prognostic factor in GBM (age), but also appears to be closely linked to chemotherapeutic responsiveness of these tumors as well. Moreover, since age is the single most dominant factor influencing the outcome of most human tumors, establishing the generality of such an immune impact on distinct tumors could substantially broaden clinical expectations associated with immune-based cancer therapies. In this context, it will be additionally important to determine whether cellular immune processes similarly influence clinical outcome and chemotherapeutic efficacy in distinct human tumors.

ACKNOWLEDGEMENTS

We thank Drs. Julie Korenberg and John S. Yu, Cedars-Sinai Research Institute, for invaluable insight in preparing the manuscript, and Dr. Janet Elashoff and Meenu Sandhu CSRI Biostatistics Core, for statistical analysis and critique.

REFERENCES

1. DeAngelis, L. M. 2001. Medical progress: Brain tumors. *N Engl J Med* 344:114.
2. Davis, F. G., V. Kupelian, S. Freels, B. McCarthy, and T. Surawicz. 2001. Prevalence estimates for primary brain tumors in the United States by behavior and major histology groups. *Neuro-oncol* 3:152.
3. Curran, W. J. J., C. B. Scott, J. Horton, J. S. Nelson, A. S. Weinstein, A. J. Fischbach, C. H. Chang, M. Rotman, S. O. Asbell, R. E. Krisch, and e. al. 1993. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. *J Natl Cancer Inst* 85:690.
4. Kreth, F. W., P. C. Warnke, R. Scheremet, and C. B. Ostertag. 1993. Surgical resection and radiation therapy versus biopsy and radiation therapy in the treatment of glioblastoma multiforme. *J Neurosurg* 78:762.
5. Quigley, M. R., N. Flores, J. C. Maroon, B. Sargent, S. Lang, and A. Elrifai. 1995. Value of surgical intervention in the treatment of glioma. *Stereotact Funct Neurosurg* 65:171.
6. Hentschel, S. J., and F. F. Lang. 2003. Current surgical management of glioblastoma. *Cancer J* 9:113.
7. Reavey-Cantwell, J. F., R. I. Haroun, M. Zahurak, R. E. Clatterbuck, R. J. Parker, R. Mehta, J. P. Fruehauf, and H. Brem. 2001. The prognostic value of tumor markers in patients with glioblastoma multiforme: analysis of 32 patients and review of the literature. *J Neurooncol* 55:195.
8. Stupp, R., and M. E. Hegi. 2003. Recent developments in the management of malignant glioma. *J Clin Oncol* 1091-9118:779.
9. Fine, H. A., K. B. G. Dear, and J. S. Loeffler. 1993. Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults. *Cancer* 71:2585.
10. Diete, S., T. Treuheit, K. Dietzmann, U. Schmidt, and C. W. Wallesch. 2001. Sex differences in length of survival with malignant astrocytoma, but not with glioblastoma. *J Neurooncol* 53:47.

11. Glick, R. P., T. Lichtor, A. Mogharbel, C. A. Taylor, and E. P. Cohen. 1997. Intracerebral versus subcutaneous immunization with allogeneic fibroblasts genetically engineered to secrete interleukin-2 in the treatment of central nervous system glioma and melanoma. *Neurosurgery* 41:898.
12. Liao, L. M., K. L. Black, R. M. Prins, S. N. Sykes, P. L. DiPatre, T. F. Cloughesy, D. P. Becker, and J. M. Bronstein. 1999. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J Neurosurg* 90:1115.
13. Yu, J. S., C. J. Wheeler, P. M. Zeltzer, H. Ying, D. N. Finger, P. K. Lee, W. H. Yong, F. Incardona, R. C. Thompson, M. S. Riedinger, W. Zhang, R. M. Prins, and K. L. Black. 2001. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res* 61:842.
14. Rosenberg, S. A., J. C. Yang, D. J. Schwartzentruber, P. Hwu, F. M. Marincola, S. L. Topalian, N. P. Restifo, M. E. Dudley, S. L. Schwarz, P. J. Spiess, J. R. Wunderlich, M. R. Parkhurst, Y. Kawakami, C. A. Seipp, J. H. Einhorn, and D. E. White. 1998. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat Med* 4:321.
15. Lee, K. H., E. Wang, M. B. Nielsen, J. Wunderlich, S. Migueles, M. Connors, S. M. Steinberg, S. A. Rosenberg, and F. M. Marincola. 1999. Increased vaccine-specific T cell frequency after peptide-based vaccination correlates with increased susceptibility to in vitro stimulation but does not lead to tumor regression. *J Immunol* 163:6292.
16. Fong, L., Y. Hou, A. Rivas, C. Benike, A. Yuen, G. A. Fisher, M. M. Davis, and E. G. Engleman. 2001. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc Natl Acad Sci U S A* 98:8809.
17. Bodey, B., B. J. Bodey, S. E. Siegel, and H. E. Kaiser. 2000. Failure of cancer vaccines: the significant limitations of this approach to immunotherapy. *Anticancer Res* 20:2665.
18. Bowles, A. P. J., and E. Perkins. 1999. Long-term remission of malignant brain tumors after intracranial infections: a report of four cases. *Neurosurgery* 44:636.

19. Douek, D. C., R. A. Vescio, M. R. Betts, J. M. Brenchley, B. J. Hill, L. Zhang, J. R. Berenson, R. H. Collins, and R. A. Koup. 2000. Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution. *The Lancet* 355:1875.
20. Okada, H., F. S. Lieberman, H. D. Edington, T. F. Witham, M. F. Wargo, Q. Cai, E. H. Elder, T. L. Whiteside, S. C. J. Schold, and I. F. Pollack. 2003. Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of recurrent glioblastoma: preliminary observations in a patient with a favorable response to therapy. *J Neuro-Oncol* 64:13.
21. Douek, D. C., R. D. McFarland, P. H. Keiser, E. A. Gage, J. M. Massey, B. F. Haynes, M. A. Polis, A. T. Haase, M. B. Feinberg, J. L. Sullivan, B. D. Jamieson, J. A. Zack, L. J. Picker, and R. A. Koup. 1998. Changes in thymic function with age and during the treatment of HIV infection. *Nature* 396:690.
22. Jamieson, B. D., D. C. Douek, S. Killian, L. E. Hultin, D. D. Scripture-Adams, J. V. Giorgi, D. Marelli, R. A. Koup, and J. A. Zack. 1999. Generation of functional thymocytes in the human adult. *Immunity* 10:569.
23. Wheeler, C. J., K. L. Black, G. Liu, H. Ying, J. S. Yu, W. Zhang, and P. K. Lee. 2003. Thymic CD8+ T cell production strongly influences tumor antigen recognition and age-dependent glioma mortality. *J Immunol* in press.
24. Lacroix, M., D. Abi-Said, D. R. Fourny, Z. L. Gokaslan, W. Shi, F. DeMonte, F. F. Lang, I. E. McCutcheon, S. J. Hassenbusch, E. Holland, K. Hess, C. Michael, D. Miller, and R. Sawaya. 2001. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg* 95:190.
25. Chandler, K. L., M. D. Prados, M. Malec, and C. B. Wilson. 1993. Long-term survival in patients with glioblastoma multiforme. *Neurosurgery* 32:716.

FOOTNOTES

¹Supported by grants from the Joseph Drown Foundation and the Maxine Dunitz Neurosurgical research fund.

²To whom reprints should be addressed:

Christopher J. Wheeler, Ph.D.

8631 W. Third Street, Suite 800E

Los Angeles, CA 90048

E-Mail: wheelerc@csbs.org

Fax: 310-423-0302

Telephone: 310-423-6646

³Abbreviations: GBM, glioblastoma multiforme; TREC, T cell receptor excision circle; SRS, stereotactic radiosurgery; qPCR, quantitative real-time PCR; KPS, Karnofsky performance status; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

TABLE I

Demographic and treatment parameters of GBM patient groups¹.

	Vaccine	Chemotherapy	Vaccine + Chemotherapy	Significance (p)
Age	53.4 ± 13	55.7 ± 10	54.0 ± 10	0.88 *
Post-vaccine Karnofsky Score	84 ± 16	-	93 ± 9	0.12 *
% male	50	38	77	0.14 †
% non-survivors	100	92	77	0.3 †
% > 2 surgeries before vaccine	58.3	-	61.5	0.43 †
% no chemo before vaccine	66.7	-	61.5	0.58 †
% no surgery after vaccine	58.3	-	53.8	1.0 †
Days from surgery to vaccine	115 ± 14	-	121 ± 13	0.94 ¶
Mean survival (months)	17.9 ± 1.7	15.9 ± 2.1	26 ± 3.7	0.047 ¶
% 2-year survival (fraction)	8.3 (1/12)	8.3 (1/12)	41.7 (5/12)	> 0.05 †; < 0.05 **
% 3-year survival (fraction)	0 (0/12)	0 (0/12)	18.2 (2/11)	> 0.05 †; < 0.01 **

¹Statistical methods: * ANOVA; † Fisher's Exact Test; ¶ Log-rank; ** Binomial distribution. Calculations of % 2- and 3-year survival excluded censored values.

TABLE II

Vaccine trial composition and distinctions¹.

	Phase I A	Phase I B	Phase II
Vaccine	7 pts	3 pts	2 pts
Vaccine + chemotherapy	1 pts	2 pts	10 pts
Antigen source	Tumor line MHC I-elution	Tumor lysate	Tumor lysate
Vaccine course	3; 2 wks apart	3; 2 wks apart	3; 2 wks apart + 1; 6 wks later
Eligibility			
Diagnosis	de novo GBM, de novo AA	de novo or recurrent GBM, AA	de novo or recurrent GBM, AA
Karnofsky score	≥ 60	≥ 60	≥ 60
Age	≥ 18 yr	≥ 18 yr	≥ 18 yr
CTL Responsiveness (GBM only)	60% *	60% †¶	40% ¶

¹CTL responsiveness was determined from 5 testable samples per trial: * Bulk CTL assay; † Elispot; ¶ IFN- γ production by qPCR.

TABLE III

Chemotherapy use¹.

Vaccine + Chemotherapy	Pt #	Drug(s)
	1	Gliadel wafers
	2	Temozolamide
	3	Temozolamide
	4	Temozolamide
	5	Temozolamide, Irinotecan
	6	Temozolamide
	7	Temozolamide, Accutane
	8	Tamoxifen
	9	Temozolamide, CCNU
	10	Temozolamide, Accutane
	11	Temozolamide, Gleevec
	12	Temozolamide, Procarbazine, CCNU, Vincristine
	13	Temozolamide, Thalidomide, Etoposide
Chemotherapy		
	1	Temozolamide
	2	Temozolamide
	3	Temozolamide, Procarbazine
	4	Temozolamide, Carboplatin, Vincristine, Procarbazine
	5	Temozolamide, BCNU, Thalidomide
	6	Temozolamide, Procarbazine
	7	Temozolamide
	8	Temozolamide, Thalidomide
	9	Gliadel wafers, Vincristine
	10	Temozolamide, Carboplatin, Vincristine
	11	BCNU, Temozolamide
	12	Gliadel wafers, Temozolamide
	13	BCNU

¹Temozolomide standard dose is 150-200 mg/m² qd x 5 days every 28 days
 BCNU 150-200 mg/m² iv q 6 weeks. Gliadel wafers are a timed-release encapsulation of BCNU.

FIGURE LEGENDS

Figure 1. A) Tumor progression (recurrence) intervals monitored for each group of GBM patients. Progression times were monitored over intervals spanning vaccination or chemotherapy and subsequently thereafter. B) Time to tumor progression in vaccine, chemotherapy and vaccine + chemotherapy groups. Tumor progression was defined as the time from first diagnosis of brain tumor (de novo GBM in all cases) to the first new scan enhancement, if verified by subsequent scans or by histology, or time from diagnosis to death due to tumor progression. Mean times to tumor progression \pm standard error are shown for each group over specific intervals, as indicated. Significance (p values) were derived from double-sided paired (initial recurrence after vaccine vs. subsequent recurrence after chemotherapy in vaccine + chemotherapy group) or unpaired double-sided T tests (all other comparisons). Initial recurrence times were identical among all three groups ($P > 0.6$). The small difference in subsequent recurrence times between vaccine and chemotherapy groups was not statistically significant ($P = 0.07$).

Figure 2. Overall survival in vaccine, chemotherapy and vaccine + chemotherapy groups. Overall survival was defined as the time from first diagnosis of brain tumor (de novo GBM in all cases) to death due to tumor progression. Kaplan-Meier survival plots with censored values in open circles are shown for each group. Broken line: chemotherapy group; solid thin line: vaccine group; solid bold line: vaccine + chemotherapy group. Survival of the vaccine group was identical to that of chemotherapy group ($p = 0.7$, log-rank). Survival of vaccine + chemotherapy group was significantly greater relative to both survival in the other two groups ($p = 0.047$, log-rank), and greater than survival in the chemotherapy group alone ($p = 0.02$, log-rank). Survival of the vaccine group tended to be lower but was not statistically different than that of the vaccine + chemotherapy group ($p = 0.05$, log-rank).

Figure 3. Tumor regression following post-vaccine chemotherapy. Relative days after diagnosis are represented by numbers under individual MRI scans, with individual patients' scans in each row. Patient A recurred 82 days after vaccine initiation; patient B recurred 147 days after vaccine initiation, was treated surgically, and recurred 227 additional days (374 days total) after vaccine initiation. An additional patient suffering tumor recurrence 35 days after vaccine initiation and treated with subsequent chemotherapy experienced objective tumor regression, but a complete array of images was not available for this individual. All scans except the pre-resection scan for patient B were performed post-contrast enhancement with gadolinium. Two of the 3 patients exhibiting objective tumor regression survived more than 2 years (730 days) post-diagnosis.

Figure 4. CD8⁺ TRECs are strongly associated with chemotherapeutic responses following vaccination. The increase in time to tumor progression in months (y-axis) was correlated in the same GBM patients with A) TRECs quantified within 50,000 purified CD8⁺ T cells from peripheral blood mononuclear cells (PBMC) collected at the time of surgery or B) patient age. Data were derived from all vaccinated GBM patients for whom chemotherapeutic response and TREC results ($n = 13$) or age ($n = 17$) were available. A related parameter, time to tumor progression after chemotherapy divided by time to tumor progression after vaccination, also correlated significantly with CD8⁺ TRECs ($r = 0.73$; $P < 0.01$), but failed to do so with patient age ($r = -0.40$; $P > 0.05$).

WE CLAIM:

1. A method for treating a brain tumor in a mammal, comprising:
administering at least one vaccination of dendritic cells to a region of the brain in said mammal; and
administering a regimen of chemotherapy to said mammal.
2. The method of claim 1, wherein said dendritic cells are autologous tumor antigen-presented dendritic cells.
3. The method of claim 1, wherein administering said at least one vaccination further comprises administering at least three vaccinations of dendritic cells.
4. The method of claim 1, wherein prior to administering said at least one vaccination, said method further comprises implementing a clinical procedure to said mammal selected from the group consisting of surgical resection of said brain tumor, radiation therapy, and combinations thereof.
5. The method of claim 1, wherein administering said at least one vaccination of dendritic cells occurs prior to administering said regimen of chemotherapy.
6. The method of claim 1, wherein said brain tumor is a glioblastoma multiforme.
7. A method of influencing chemosensitivity of a mammal with a brain tumor, comprising:
providing a vaccine including dendritic cells; and
administering said vaccine to said mammal.
8. The method of claim 7, wherein said dendritic cells are autologous tumor antigen-presented dendritic cells.
9. The method of claim 7, wherein said brain tumor is a glioblastoma multiforme.

ABSTRACT

Malignant gliomas are the most common primary adult brain tumor. Prognosis and outcome of the most common of these incurable tumors, glioblastoma multiforme (GBM), remains dismal despite therapeutic advances. Thus, novel therapeutic strategies are needed to treat this disease. The development of immune-based therapies for various cancers including malignant glioma has been heralded with much hope and optimism, although the ability of current cancer immunotherapies by themselves to elicit objective clinical improvements for most patients has not been realized. Synergy of immunotherapies with more conventional treatments has not been examined. Clinical outcomes were monitored in 25 vaccinated (13 with and 12 without subsequent chemotherapy) and 13 non-vaccinated GBM patients receiving chemotherapy. Overall survival from brain tumor diagnosis was ascertained and times to objective tumor progression monitored by serial MRI scans every 2-3 months. T cell receptor excision circle (TREC) content within CD8⁺ T cells was determined in patients responding to chemotherapy. Vaccinated patients receiving subsequent chemotherapy exhibited significantly longer survival relative to those receiving vaccinations without subsequent chemotherapy ("vaccination alone"). Moreover, the former group exhibited significantly delayed tumor progression following chemotherapy relative to patients receiving vaccination or chemotherapy alone, despite exhibiting identical tumor progression times initially (i.e., following vaccination). Additional demographic and treatment parameters failed to account for this difference in outcomes. CD8⁺ TRECs correlated precisely with increased tumor progression times after chemotherapy, independently corroborating a cellular immune influence on GBM chemosensitivity. We propose that therapeutic vaccination synergizes with subsequent chemotherapy to elicit tangible clinical benefits for GBM patients.

Figure 1A

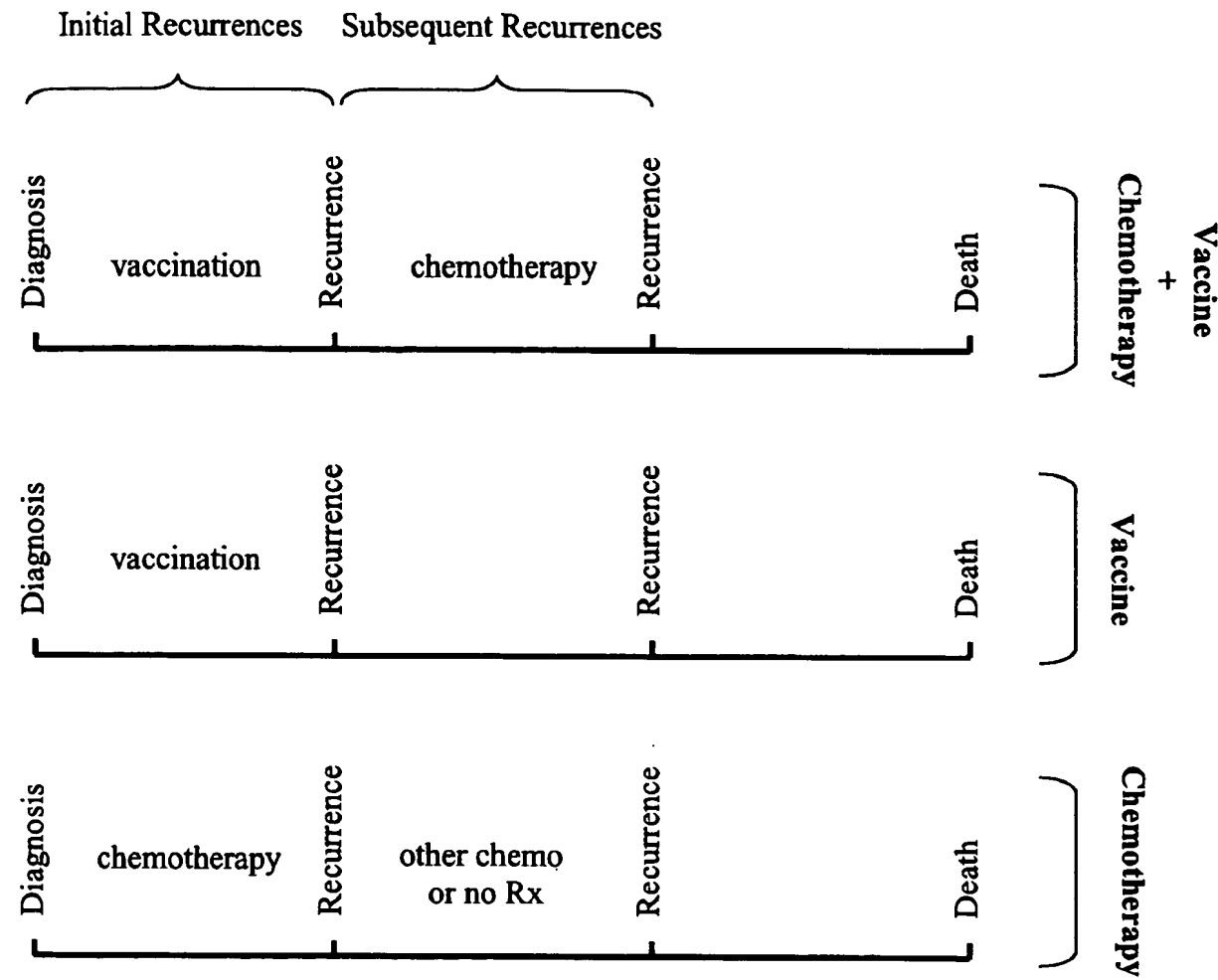


Figure 1B

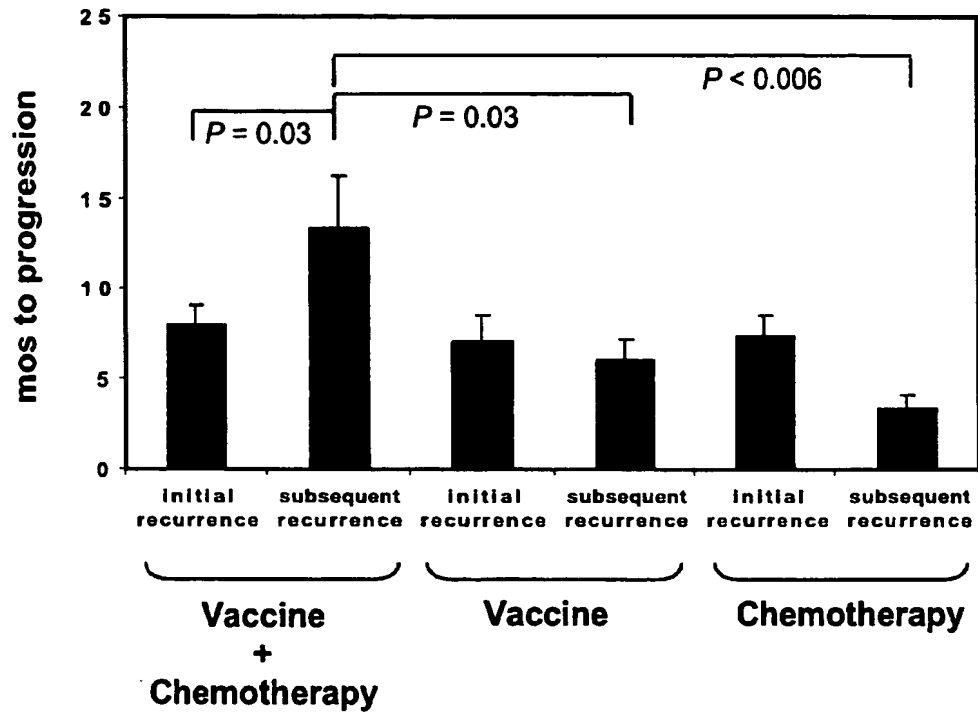


Figure 2

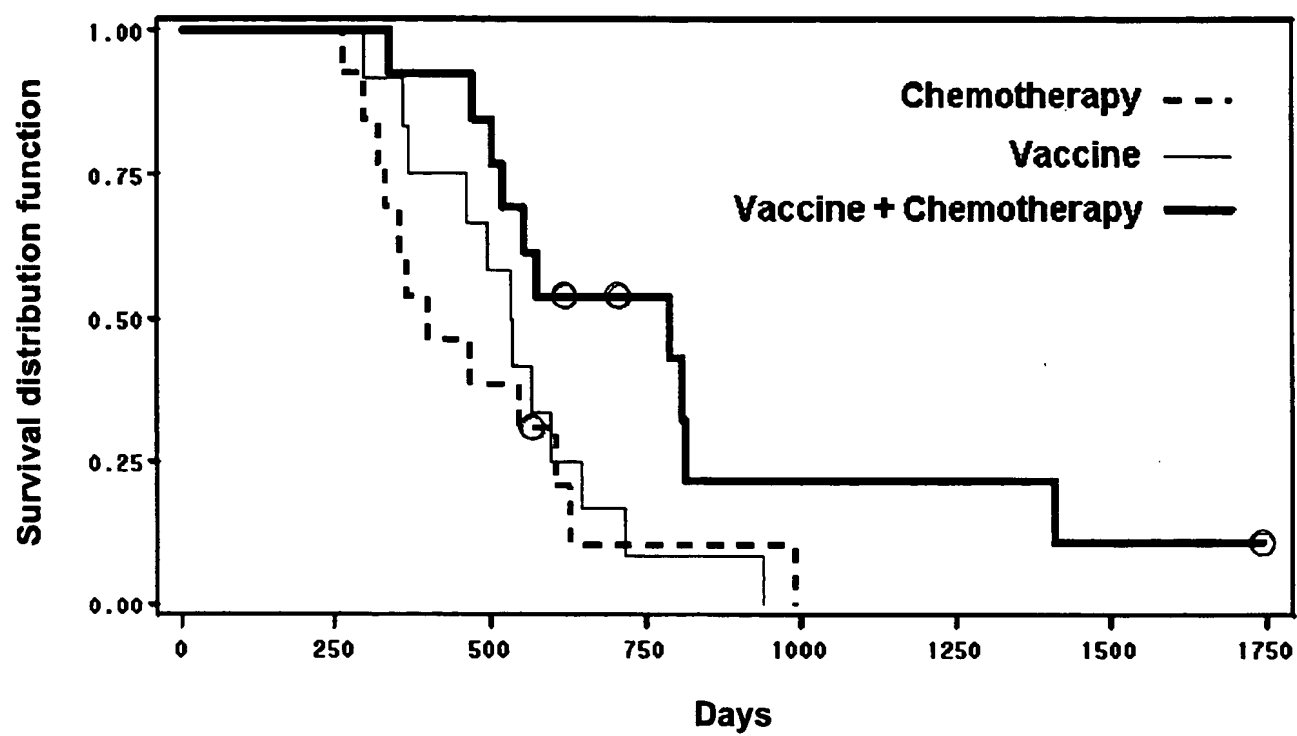


Figure 3

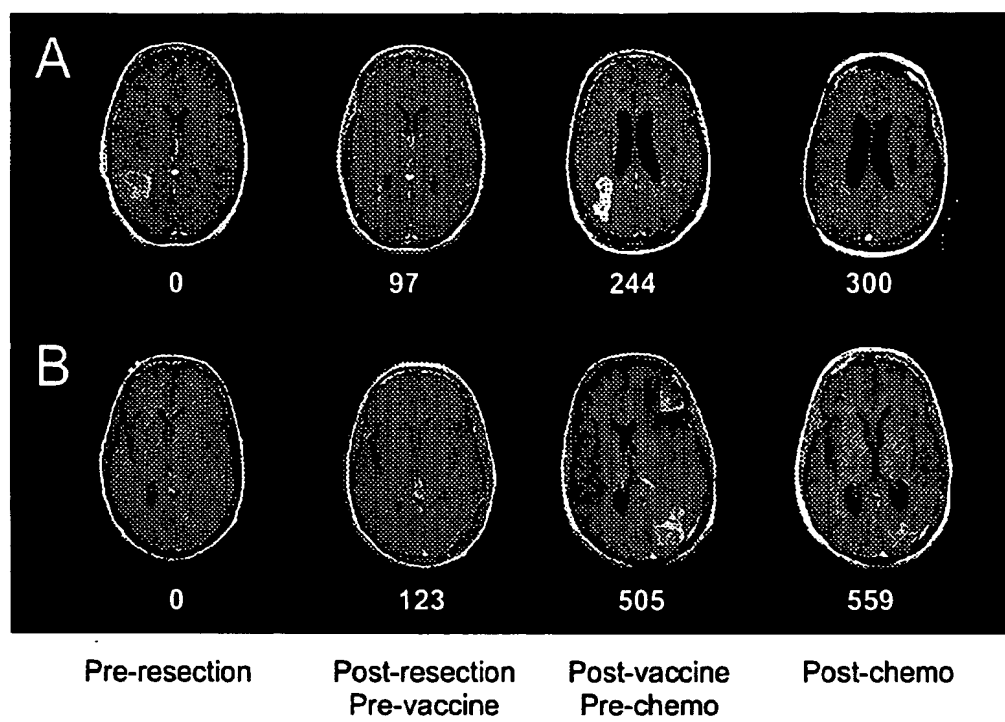
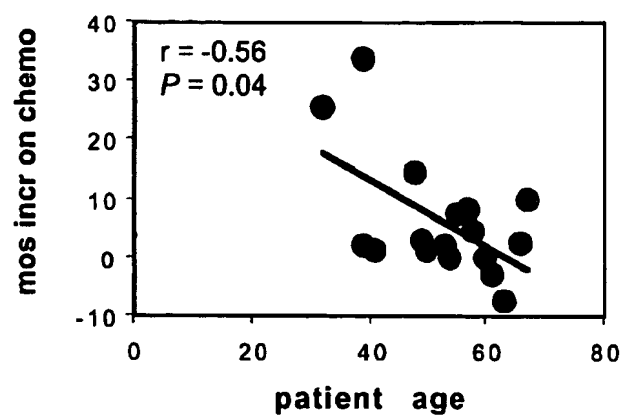
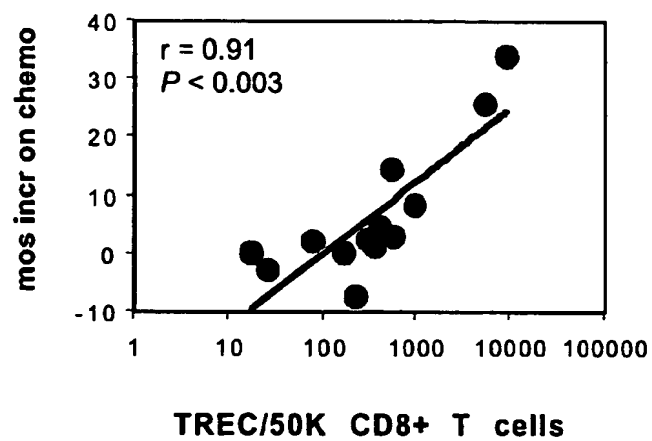


Figure 4

A



B



Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/034761

International filing date: 20 October 2004 (20.10.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/513,040
Filing date: 21 October 2003 (21.10.2003)

Date of receipt at the International Bureau: 09 December 2004 (09.12.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



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